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I, LEANNE MYNOTT, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 1054 for a patent by BIOTA SCIENTIFIC MANAGEMENT PTY LTD filed on 18 June 1999.

WITNESS my hand this
Thirtieth day of June 2000

LEANNE MYNOTT
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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Biota Scientific Management Pty Ltd

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Antiviral Agents"

The invention is described in the following statement:

ANTIVIRAL AGENTS

This invention relates to antiviral agents, in particular to compounds useful in the treatment of infections caused by Picornaviridae, such as human rhinovirus (HRV) and methods for their preparation. The invention also relates to the use of these compounds in the treatment of picornavirus infections and to intermediates useful in the preparation of these compounds. The compounds of the invention are especially suitable for use in the treatment of HRV and accordingly it will be convenient to describe the invention in connection with these viruses. However it is to be understood that the invention is also applicable to other viruses of the Picornavirus family.

Human rhinovirus are a member of the genus *Rhinovirus* of the picornavirus family and are believed to be responsible for between 40 and 50% of common cold infections. Human rhinoviruses comprise a group of over 100 serotypically distinct viruses and accordingly antiviral activity for multiple serotypes and potency are considered to be equally important factors in drug design.

Two cellular receptors have been identified to which almost all typed HRVs bind. The major group, which comprises 91 of the more than 100 typed serotypes, binds to the intracellular adhesion molecule-1 (ICAM-1) while the minor group, which comprises the rest of typed serotypes with the exception of HRV87, binds to the low density lipoprotein receptor family of proteins.

Another genus of the Picornaviridae family is represented by the Enteroviruses. This genus includes polioviruses 1-3, coxsackieviruses A (23 serotypes) and B(6 serotypes), echoviruses (31 serotypes) and numbered enteroviruses 68-71. The clinical syndromes caused by enteroviruses include poliomyelitis, meningitis, encephalitis, pleurodynia, herpangina, hand foot and mouth disease, conjunctivitis, myocarditis and neonatal diseases such as respiratory illnesses and febrile illnesses.

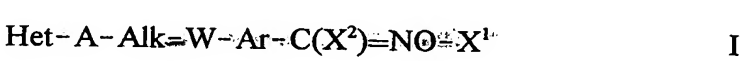
Viruses of the Picornavirus family are characterised by a single stranded (+) RNA genome encapsidated by a protein shell (or capsid) having pseudo icosahedral symmetry. The surface of the capsid contains "canyons" which surround each of the icosahedral fivefold axes and it is believed that the cellular receptors bind to residues on the canyon floor.

A hydrophobic pocket lies underneath the canyon within which a number of antiviral compounds are capable of binding, sometimes with consequential conformational changes. Some of these compounds have been shown to inhibit the uncoating of HRVs and, for some of the major receptor group viruses, inhibition of cell receptor binding has also been demonstrated. It has also been shown that when a compound is bound within the hydrophobic capsid pocket, HRVs are more stable to denaturation by heat or acids.

Examples of antipicornoviral compounds believed to act by binding within the hydrophobic pockets of the picornavirus capsid are described in US Patents 4,992,433, 5,100,893, 5,070,090 and Australian Patent No. 628172. One compound which has been the subject of recent human clinical trials is ethyl 4-[2-[1-(6-methyl-3-pyridazinyl)-4-piperidinyl]-ethoxy]benzoate, otherwise known as "Pirodavir". ("Intranasal Pirodavir (R77,975) Treatment of Rhinovirus Colds" F.G. Hayden, et al., *Antimicrobial Agents and Chemotherapy*, **39**, 290-294, 1995).

A novel class of antiviral compounds has now been discovered which has been found to exhibit particularly favourable antipicornaviral properties.

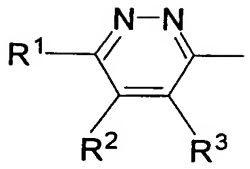
Accordingly the present invention provides a compound of formula (I)



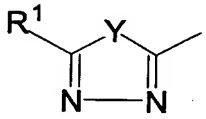
I

and pharmaceutically acceptable salts and esters thereof where

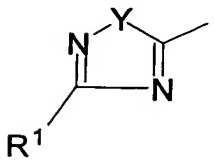
Het is a radical of formula



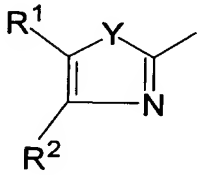
(a-1)



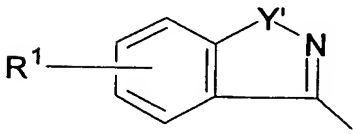
(a-2)



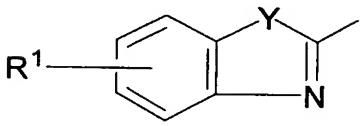
(a-3)



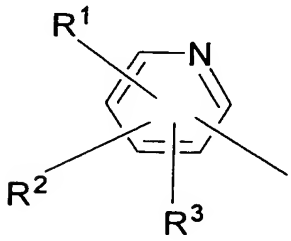
(a-4)



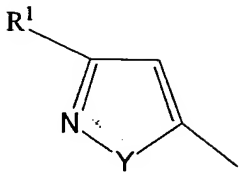
(a-5)



(a-6)



(a-7)



(a-8)

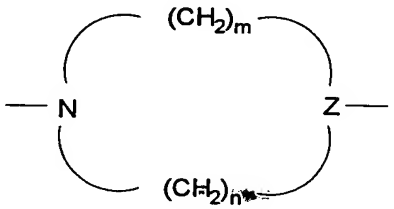
wherein R^1 is hydrogen, C_{1-6} alkyl, halo, hydroxy, mercapto, trifluoromethyl, amino, mono or di(C_{1-6} alkyl)amino, cyano, C_{1-6} alkoxy, C_{1-6} haloalkoxy, aryloxy, C_{1-6} alkylthio, arylthio, C_{1-6} alkylsulphinyl, C_{1-6} alkylsulphonyl, arylsulphinyl, arylsulphonyl, C_{1-6} alkyloxycarbonyl, C_{1-6} alkylcarbonyl or aryl;

R^2 and R^3 are each independently selected from hydrogen, C_{1-6} alkyl, C_{1-6} alkoxy, halo or, in radicals (a-1) and (a-7), R^2 and R^3 combined may represent a bivalent radical of formula $-CH=CH-CH=CH-$;

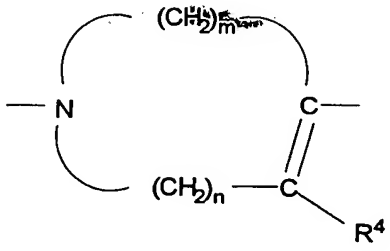
Y is O or S; and

Y′ is O, S, SO or SO_2;

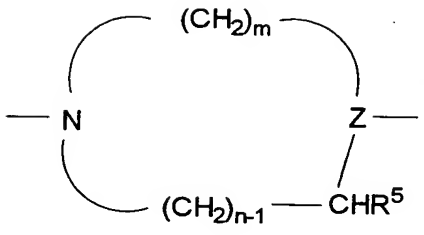
A is O, S, NH, N(C_{1-6} alkyl) CH_2O, a bond or a bivalent heterocyclic radical of the formula



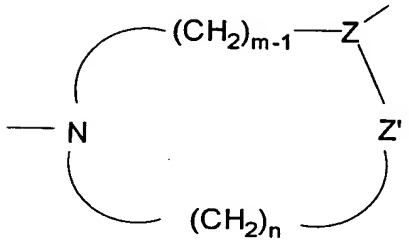
(b-1),



(b-2),



(b-3), or



(b-4)

where one or more of the carbon atoms within the radicals (b-1) to (b-4) may be optionally substituted with C_{1-6} alkyl or two carbon atoms in the radicals (b-1) to (b-4) may be bridged with a C_{2-4} alkylene radical, m and n are each independently integers of 1 to 4 inclusive with the proviso that the sum of m and n in radicals (b-1) to (b-4) is 3, 4 or 5;

Z is N or CR^6 where R^6 is hydrogen, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy or amino;

Z′ is O, S, CHR^7 or NR^8 where R^7 is hydrogen, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy or amino and R^8 is hydrogen or C_{1-6} alkyl;

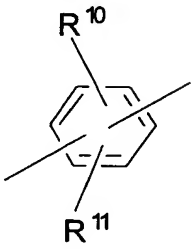
R^4 is hydrogen or C_{1-6} alkyl; and

R^5 is hydrogen, hydroxy, C_{1-6} alkyl or C_{1-6} alkoxy;

Alk is C_{1-7} alkylene or a direct bond;

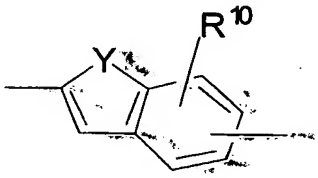
W is O, S, OCH_2, a direct bond or NR^9 where R^9 is hydrogen or C_{1-6} alkyl;

Ar is a bivalent aromatic radical of the formula

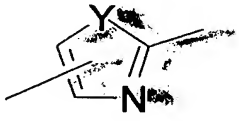


(c-1)

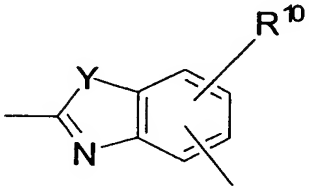
(c-2)



(c-3)



(c-4)



(c-5)

(c-6)

where Y is as defined above;

R^{10} and R^{11} are each independently hydrogen, C_{1-6} alkyl, hydroxy C_{1-6} alkyl, halo, amino, cyano, nitro, C_{1-6} alkoxy, hydroxy, C_{1-6} alkylthio, or trifluoromethyl;

X^1 is C_{1-6} alkyl, C_{3-6} alkenyl, C_{3-6} haloalkenyl, C_{3-6} alkynyl, C_{3-6} haloalkynyl or C_{1-6} alkyl, substituted by halo, cyano, nitro, hydroxy, aryl, C_{1-4} alkoxy, C_{2-6} alkoxyalkoxy or C_{1-4} alkylthio;

X^2 is hydrogen, cyano, C_{1-4} alkyl or C_{1-4} haloalkyl.

As used herein the term C_{1-6} alkyl as used alone or as part of a group such as "di(C_{1-6} alkyl)amino" refers to straight chain, branched or cyclic alkyl groups having from 1 to 6 carbon atoms. Examples of such alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, cyclopentyl and cyclohexyl.

As used herein the term "halo" as used alone or as part of a group such as "C_{3-6} halo alkenyl" refers to fluoro, chloro, bromo and iodo groups.

As used herein the terms "C_{1-6} alkoxy" and "C_{1-6} alkyloxy" refer to straight chain or branched alkoxy. Examples of C_{1-6} alkoxy include methoxy, ethoxy, n-propoxy, isopropoxy, and the different butoxy isomers.

As used herein the term "C_{3-6} alkenyl" refers to groups formed from C_{3-6} straight chain, branched or cyclic alkenes. Examples of C_{3-6} alkenyl include allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1,3-butadienyl, 1-4,pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl and 1,4-cyclohexadienyl.

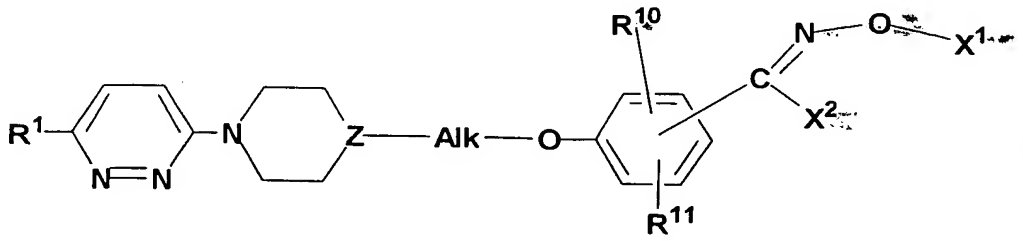
As used herein the term "C_{3-6} alkynyl" refers to groups formed from C_{3-6} straight chain or branched groups as previously defined which contain a triple bond. Examples of C_{3-6}

alkynyl include 2,3-propynyl and 2,3- or 3,4-butynyl.

As used herein the term "aryl" refers to aromatic rings or ring systems. The aromatic rings may be carbocyclic, heterocyclic or pseudo aromatic, and may be mono- or bi-cyclic ring systems. The aromatic rings or ring systems are generally composed of 3 to 15 carbon atoms and, in the case of hetero aromatic rings, may contain one or more heteroatoms selected from N, S and O. Examples of suitable aryl groups include but are not limited to phenyl, biphenyl, naphthyl, tetrahydronaphthyl, pyridinyl, thiophenyl, benzothiophenyl, furyl, isobenzofuranyl, pyrrolyl, imidazolyl, pyrazolyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, indolizinyl, isoindolyl, purinyl, oxazolyl, thiazolyl, isothiazolyl, isooxazolyl, triazinyl, triazolyl, tetrazolyl and the like, each of which may be optionally substituted with C_{1-6} alkyl, C_{1-6} alkoxy, C_{3-6} alkynyl, C_{3-6} alkynyl, halo, hydroxy, mercapto, trifluoromethyl, amino, cyano or mono or di(C_{1-6} alkyl) amino. The term "pseudoaromatic" refers to a ring system which is not strictly aromatic, but which is stabilized by means of delocalization of electrons and behaves in a similar manner to aromatic rings. Examples of pseudoaromatic rings include but are not limited to furan, thiophene, pyrrole and the like.

Preferred aryl groups include phenyl, pyridinyl, pyridazinyl, pyrazinyl, pyrimidinyl, 1,2,4-triazinyl, furyl, thiophenyl thiazolyl, isothiazolyl, isoxazolyl, 1,2,4-triazolyl, oxazolyl, imidazolyl, pyrazolyl, 1,4-benzothiazinyl, indolyl and benzofuranyl.

A particular group of compounds of the invention has the formula II:



II

wherein:

R^1 is hydrogen, C_{1-4} alkyl, halo, hydroxy, mercapto, trifluoromethyl, amino, mono or di(C_{1-4} alkyl)amino, cyano, C_{1-4} alkoxy, C_{1-4} haloalkoxy, aryloxy, C_{1-4} alkylthio, or aryl;

Z is CH or N;

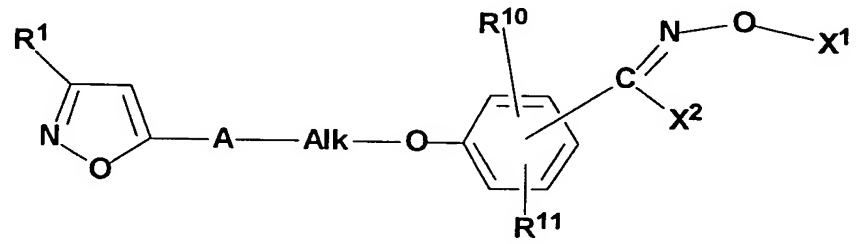
Alk is C_{1-6} alkylene;

R^{10} and R^{11} are each independently hydrogen, C_{1-4} alkyl, C_{1-4} alkoxy, halo, hydroxy;

X^1 is C_{1-6} alkyl, C_{3-6} alkenyl, C_{3-6} haloalkenyl, C_{3-6} alkynyl, C_{3-6} haloalkynyl or C_{1-6} alkyl optionally substituted by halo, cyano, nitro, hydroxy, aryl, C_{1-4} alkoxy or C_{1-4} alkylthio; and

X^2 is hydrogen, cyano, C_{1-4} alkyl or C_{1-4} haloalkyl.

Another particular set of compounds of the invention have the formula III:



III

wherein:

R^1 is hydrogen, C_{1-4} alkyl, halo, hydroxy, mercapto, trifluoromethyl, amino, mono or di(C_{1-4} alkyl)amino, cyano, C_{1-4} alkoxy, C_{1-4} haloalkoxy, aryloxy, C_{1-4} alkylthio, or aryl;

A is a bond or CH_2O;

Alk is C_{1-7} alkylene;

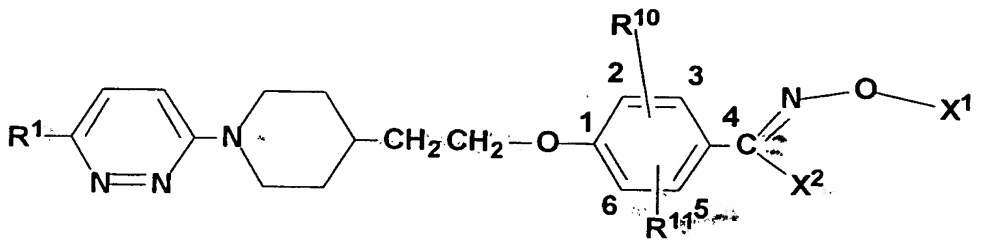
R^{10} and R^{11} are each independently hydrogen, C_{1-4} alkyl, C_{1-4} alkoxy, halo, hydroxy;

X^1 is C_{1-6} alkyl, C_{3-6} alkyenyl, C_{3-6} haloalkenyl, C_{3-6} alkynyl, C_{3-6} haloalkynyl or C_{1-6} alkyl optionally substituted by halo, cyano, nitro, hydroxy, aryl, C_{1-4} alkoxy or C_{1-4} alkylthio; and

X^2 is hydrogen, cyano, C_{1-4} alkyl or C_{1-4} haloalkyl.

Examples of specific compounds within the scope of the present invention are shown in Tables 1, 2 and 3 below.

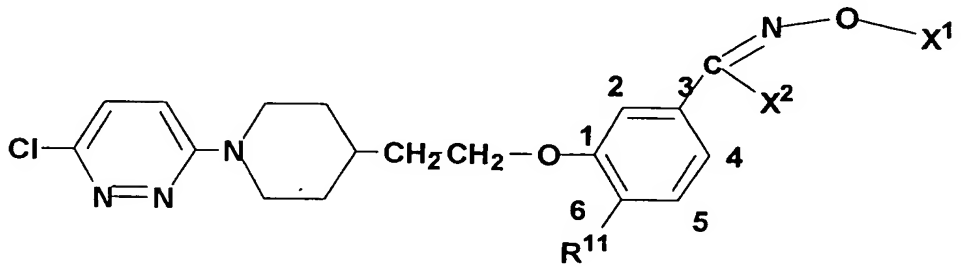
Table 1



Compound Number	Substituent R^1	Substituents R^{10}; R^{11}	Group X^1	Group X^2
1	Cl	H	CH_2CH_3	H
2	Me	H	CH_2CH_3	H
3	Cl	H	CH_2CH_3	CH_3
4	Me	H	CH_2CH_3	CH_3
5	Cl	2-(OMe)	CH_2CH_3	H
6	Cl	2,6-$(OMe)_2$	CH_2CH_3	CH_3
7	Me	H	CH_3	H
8	Cl	H	CH_3	H
9	Me	H	CH_3	CH_3
10	Cl	H	CH_3	CH_2CH_3
11	Cl	H	$CH_2CH_2CH_3$	H
12	Cl	H	$CH(CH_3)_2$	H
13	Cl	H	$CH_2CH=CH_2$	H
14	Cl	H	CH_2CN	H
15	Cl	H	$CH_2C_6H_5$	H

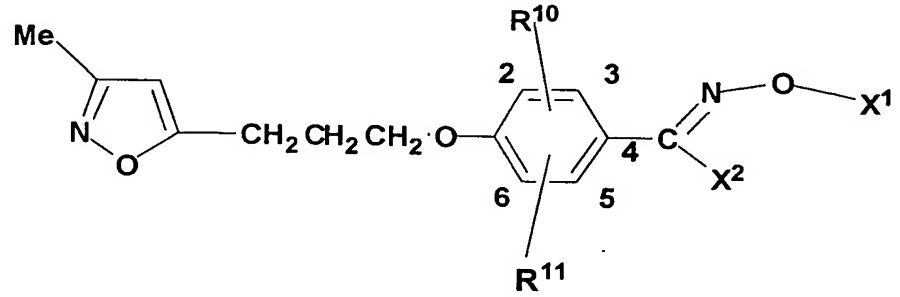
18	Cl	2,6-$(Me)_2$	CH_3	H
19	Cl	2-(OMe)	CH_3	CH_3
20	Cl	H	$(CH_2CH_2O)_2CH_2CH_3$	H
21	CF_3CH_2O	H	CH_2CH_3	H

Table 2



Compound number	Substituent R^{11}	X^1	X^2
16	H	CH_3	H
17	6-OMe	CH_3	H

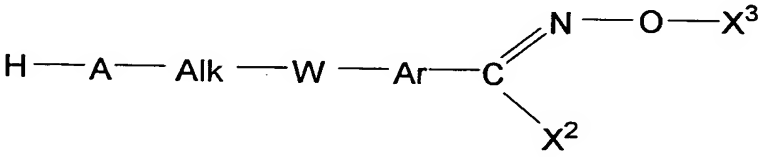
Table 3



Compound number	Substituents R^{10}, R^{11}	X^1	X^2
22	2,6-$(Me)_2$	CH_3	H
23	2,6-$(Me)_2$	$CH_2CH{=}CH_2$	H
24	2,6-$(Me)_2$	CH_2CH_3	H
25	2,6-$(Me)_2$	$CH_2C_6H_5$	H

The compounds of the present invention may be prepared using methods analogous to those described in the prior art. For example, compounds in which the Het radical is of formula (a-1) may be prepared using methodology analogous to the processes described in US Patents 4,992,433, 5,112,825 and 5,100,893. Similarly, compounds in which Het is (a-2), (a-3), (a-4), (a-5) or (a-6) may be prepared using methodology similar to that described in US Patent 5,070,090 and Australian patent No. 629172, and compounds in which Het is (a-7) or (a-8) may be prepared in accordance with methodology similar to that described in US Patent 5,364,865.

In one method the compounds of the present invention are prepared via an intermediate of formula IV:



IV

where A, Alk, W, Ar and X^2 are as described above, and X^3 is X^1 or an oxime protecting group.

This intermediate, may be prepared using methodology similar to that described in US Patent 5,231,184. In one example intermediates of formula IV, when W is O, are prepared by the reaction of compounds of the formula P-A-Alk-OH or P-A-Alk-L with hydroxy aromatic compounds of formula V.

$$HO\text{-}Ar\text{-}C(X^2)=NO\text{-}X^3 \qquad V$$

where Ar, X^1, X^2 and X^3 are as defined above, P is H or a protecting group, and L is a leaving group. Removal of the protecting group P in the reaction product affords the reactive intermediates of formula IV.

Examples of suitable protecting groups P in compounds of formula P-A-Alk-OH or P-A-Alk-L include benzyl or acyl moieties which can be introduced and removed by standard methods (see "Protective Groups in Organic Synthesis" Theodora Green, Wiley Interscience, 1981).

The intermediate of formula IV may be reacted with a compound of formula Het-L, where Het is as defined above and L is a suitable leaving group, optionally followed by deprotection and/or conversion of X^3 to X^1, to afford a compound of formula I. This N-alkylation reaction can be conducted using procedures known to the art, such as under the conditions described in US Patent 5,231,184 for performing analogous N-alkylations.

The intermediates of formula IV are novel and represent a further aspect of the present invention.

Examples of suitable leaving groups include halogen, such as fluoro, chloro, bromo and iodo, and halogen-like groups such as p-toluenesulphonyloxy and methanesulphonyloxy.

Another method for the preparation of the compounds of formula I involves the addition of an alkoxyamine H_2NOX^1 to an aldehyde or ketone of formula VI

$$Het\text{-}A\text{-}Alk\text{-}W\text{-}Ar\text{-}C(=O)X^2 \qquad VI$$

where Het, A, Alk, W, Ar X^1 and X^2 are as defined for formula I above. This reaction is carried out using standard conditions, such as in an aqueous or alcoholic solvent at ambient

temperature or with warming.

A further method of forming the compounds of the invention involves reaction of a carbonyl compound of formula VI with hydroxylamine to give of an oxime of formula VII,

$$\text{Het-A-Alk-W-Ar-C(=NOH)}X^2 \qquad \text{VII}$$

which is then O-alkylated with a compound L-X^1, where L is a leaving group and X^1 is as defined for formula I.

An additional method of preparing certain compounds of the invention of formula Ia (Compounds for formula I where W = O) involves condensing an oxime ether of formula VIII with a suitable precursor of formula (IX)

$$\text{HO-Ar-C}(X^2)\text{=NO-}X^1 \quad (\text{VIII}) \;+\; \text{Het-A-Alk-OH} \quad (\text{IX}) \;\rightarrow\; \text{Het-A-Alk-O-Ar-C}(X^2)\text{=N-O-}X^1 \quad (\text{Ia})$$

using Mitsunobu Reaction conditions (see Chemical Syntheses, Vol. 42, p 335, 1992) and where Het, A, Alk, Ar, X^1 and X^2 are as defined for formula I.

Methods for the preparation of intermediate carbonyl compounds of formulae VI have been described in the chemical literature (US Patents 4,992,433, 4,451,476). Compounds of formula VIII are well known in the chemical literature (eg German Patent DE 3,601,564). Several references, including US Patents 5,112,825 and 5,242,924 describe methods for the preparation of various compounds of formula IX.

The compounds of the present invention are useful in the prevention or treatment of picornoviral infections in mammals, particularly humans.

The picornavirus infection may be caused by any virus of the family Picornaviridae. Representative family members include human rhinoviruses, polioviruses, enteroviruses including coxsackieviruses and echoviruses, hepatovirus, cardioviruses, apthovirus, hepatitis A and other picornaviruses not yet assigned to a particular genus, including one or more of the serotypes of these viruses. Preferably the invention is used in the prevention or treatment of infection caused by one or more serotypes of rhinovirus.

Without wishing to be limited by theory it is believed that the oxime ether moiety of the compound of formula I may be involved in hydrogen bonding with an asparagine residue generally present near the opening of the hydrophobic pocket and that this interaction enhances the binding of the compounds in the capsid pocket, relative to the prior art compounds. It is further believed that the oxime ether bond may be more resistant to hydrolysis than the ester bond of pirodavir, and that this may allow more flexibility in the methods of administration of the compound to the site of activity, than available for readily hydrolysable pirodavir. In particular it may allow oral administration of the compounds or reduce metabolism in the nasal mucosa following topical administration.

The invention also provides the use of a compound of formula I in the manufacture of a medicament for the treatment of picornavirus infection.

While it is possible that, for use in therapy, a compound of the invention may be administered as the neat chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

In view of the general lipophilic nature of the compounds they are particularly suitable to oral forms of administration, however other forms of administration are also envisaged.

The invention thus further provides pharmaceutical formulations comprising a compound of the invention or a pharmaceutically acceptable salt or derivative thereof together with

one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The compounds of this invention may also be useful in combination with known anti-viral or anti-retroviral agents or other pharmaceuticals used in the treatment of viral infections. Representative examples of these additional pharmaceuticals include immunomodulators, immunostimulants, antibiotics and anti-inflammatory agents. Exemplary anti-viral agents include zanamivir, rimantidine, amantidine, ribavirin, AZT, 3TC, (-) FTC, acyclovir, famciclovir, penciclovir, ddI, ddC, ganciclovir, saquanivir, loviride, other non-nucleotide reverse transcriptase (RT) inhibitors and protease inhibitors, antiviral and antireceptor antibodies and receptor analogues, such as ICAM-1. Exemplary immunomodulators and immunostimulants include various interleukins, cytokines and antibody preparations. Exemplary antibiotics includes antifungal agents and antibacterial agents. Exemplary anti-inflammatory agents include glucocorticoids and non-steroidal anti-inflammatory compounds.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The compounds of the invention, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of pharmaceutical compositions and unit dosages thereof, and in such form may be employed as solids, such as tablets or filled capsules, or liquids such as solutions, suspensions, emulsions, elixirs, or capsules filled with the same, all for oral use, in the form of suppositories for rectal administration; or in the form of sterile injectable solutions for parenteral (including subcutaneous) use. Such pharmaceutical compositions and unit dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional

active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed. Formulations containing ten (10) milligrams of active ingredient or, more broadly, 0.1 to one hundred (100) milligrams, per tablet, are accordingly suitable representative unit dosage forms. The compounds of the present invention can be administrated in a wide variety of oral and parenteral dosage forms. It will be obvious to those skilled in the art that the following dosage forms may comprise, as the active component, either a compound of the invention or a pharmaceutically acceptable salt of a compound of the invention.

For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component.

In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired.

The powders and tablets preferably contain from five or ten to about seventy percent of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term preparation" is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component, with

or without carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid forms suitable for oral administration.

For preparing suppositories, a low melting wax, such as admixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogenous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water-propylene glycol solutions. For example, parenteral injection liquid preparations can be formulated as solutions in aqueous polyethylene glycol solution.

The compounds according to the present invention may thus be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, prefilled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavours, stabilizing and thickening

agents, as desired.

Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, or other well known suspending agents.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavours, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

For topical administration to the epidermis the compounds according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case of a dropper or pipette, this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray, this may be achieved for example by means of a metering atomising

spray pump. To improve nasal delivery and retention the compounds according to the invention may be encapsulated with cyclodextrins, or formulated with their agents expected to enhance delivery and retention in the nasal mucosa.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the active ingredient is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the active ingredients may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of, e.g., gelatin, or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the compound will generally have a small particle size for example of the order of 5 to 10 microns or less. Such a particle size may be obtained by means known in the art, for example by micronization.

When desired, formulations adapted to give sustained release of the active ingredient may be employed.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active

component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

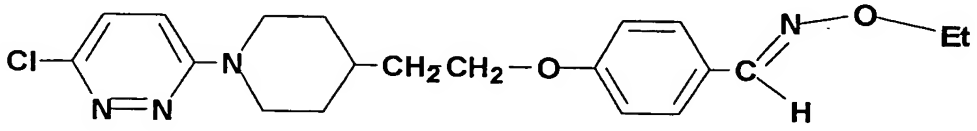
Liquids or powders for intranasal administration, tablets or capsules for oral administration and liquids for intravenous administration are preferred compositions.

The invention will now be described with reference to the following examples which illustrate some preferred aspects of the present invention. However it is to be understood that the particularity of the following description of the invention is not to supersede the generality of the preceding description of the invention.

EXAMPLES

Example 1

Preparation of 4-{2-[1-(6-Chloro-3-pyridazinyl)-4-piperidinyl]ethoxy}benzaldehyde O-ethyloxime (Compound 1)



4-{2-[1-(6-Chloro-3-pyridazinyl)-4-piperidinyl]ethoxy}benzaldehyde (Intermediate IIa) was prepared from 4-hydroxybenzaldehyde and 2-[1-(6-Chloro-3-pyridazinyl)-4-piperidinyl]ethanol using a Mitsunobu Reaction and following the methods described in US Patent 4,992,433. The aldehyde (60mg, 0.17 mmol) was dissolved in ethanol (5 ml) with stirring at room temperature and a solution of ethoxyamine in water (0.5 ml, 50% $EtONH_2$) was added. The reaction was stirred at room temperature for 2 days and then concentrated on a rotary evaporator and the residue was purified by chromatography (silica gel 18g) using

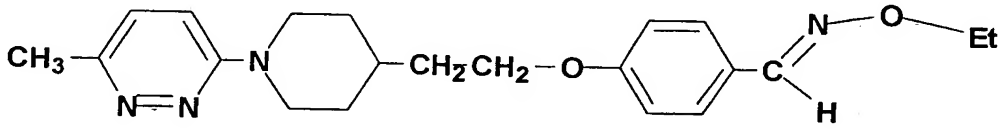
chloroform as eluent. The first compound to elute was the O-ethyloxime (1) which was obtained as a pale cream solid (30mg, 44%).

^{1}H nmr spectrum ($CDCl_3$) δ (ppm): 1.20-1.40 (m, 5H); 1.65-1.95 (m, 5H); 2.95 (bt, 2H); 4.05 (t, 2H); 4.18 (q, 2H); 4.35 (bd, 2H); 6.87 (d, 2H); 6.90 (d, 1H); 7.16 (d, 1H); 7.52 (d, 2H); 8.02 (s, 1H).

Mass Spectrum (ESI) 389 $(M+1)^+$

Example 2

Preparation of 4-{2-[1-(6-Methyl-3-pyridazinyl)-4-piperidinyl]ethoxy}benzaldehyde O-ethyloxime (Compound 2)



(a) A solution of 4-hydroxybenzaldehyde (100mg, 0.88mmol) and 50% O-ethylhydroxylamine aqueous solution (0.6ml, 4.9mmol) in dioxane (2 ml) was stirred under an atmosphere of argon at room temperature for 16 hours and then heated at 90-100° for 3 hours when thin layer chromatography (silica, dichloromethane) indicated the reaction was complete. The solution was evaporated to dryness and the residue partitioned between ethyl acetate (20 ml) and water (5 ml). The organic layer was washed with water, dried (Na_2SO_4) and evaporated to afford 4-hydroxybenzaldehyde O-ethyloxime (125mg, 92.5%).

(b) To a solution of 4-hydroxybenzaldehyde O-ethyloxime (49mg, 0.4mmol), 2-[1-(6-methyl-3-pyridazinyl)-4-piperidinyl]ethanol (88mg, 0.4mmol) and triphenylphosphine (115mg, 0.44mmol) in dry tetrahydrofuran (THF) (5 ml) under argon at room temperature was added dropwise diisopropylazodicarboxylate (89mg, 0.44mmol) in dry THF. The resulting solution was stirred at 20° for 24 hours and the solvent was then removed on a rotary evaporator. The residue was

chromatographed on silica gel, using firstly ethyl acetate/hexane (1:1) as eluent to give the product, Compound 2 as a white solid (103mg, 70%).

^{1}H nmr spectrum ($CDCl_3$) δ (ppm): 1.20-1.40 (m, 5H); 1.65-1.95 (m, 5H); 2.53 (s, 3H); 2.95 (bt, 2H); 4.05 (t, 2H); 4.18 (q, 2H); 4.35 (bd, 2H); 6.88 (d, 2H); 6.90 (d, 1H); 7.05 (d, 1H); 7.52 (d, 2H); 8.02 (s, 1H).
Mass Spectrum (ESI) 369 $(M+1)^+$

Example 3

The Compound No's 5, 8, 11, 12, 13, 14, 15, 20, 21 and 22 to 25 from Tables 1 and 3 of the invention were prepared using essentially the same method as described in Example 1 for Compound 1, using the appropriate carbonyl compound and alkoxyamine. The compounds were purified by chromatography on silica gel and characterised by their nuclear magnetic resonance (nmr) spectra and Mass Spectral (MS) data. For convenience the nmr and MS data are recorded in Table 4 below.

Table 4

Compound Number	MS data (ESI)	NMR data: Proton Chemical Shift, δ in ppm ($CDCl_3$)
5	419 (M+1)	1.20-1.40(m, 5H); 1.65-1.95(m, 5H); 3.0(bt, 2H); 3.89(s, 3H); 4.08(t, 2H); 4.21(q, 2H); 4.35(bd, 2H); 6.85(d, 1H); 6.92(d, 1H); 7.0(dd, 1H); 7.15(d, 2H); 7.24(d, 1H); 8.02(s, 1H)
8	375 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 3.93(s, 3H); 4.05(t, 2H); 4.40(d, 2H); 6.88(d, 2H); 7.0(d, 1H); 7.24(d, 1H); 7.51(d, 2H); 8.02(s, 1H)
11	403 (M+1)	0.95(t, 3H); 1.30(m, 2H); 1.65-1.95(m, 5H); 1.77(t, 2H); 2.95(t, 2H); 3.92-4.20(m, 4H); 4.35(d, 2H); 6.85(d, 2H); 6.90(d, 1H); 7.20(d, 1H); 7.50(d, 2H); 8.07(s, 1H)

12	403 (M+1)	1.25(d, 6H); 1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 4.05(t, 2H); 4.35(d, 2H); 4.42(m, 1H); 6.85(d, 2H); 6.90(d, 1H); 7.20(d, 1H); 7.51(d, 2H); 8.05(s, 1H).
13	401 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 4.05(t, 2H); 4.35(d, 2H); 4.66(d, 2H); 5.25(dd, 1H); 5.38(dd, 1H); 6.08(m, 1H); 6.85(d, 2H); 6.90(d, 1H); 7.20(d, 1H); 7.50(d, 2H); 8.13(s, 1H)
14	400 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 4.05(t, 2H); 4.35(d, 2H); 4.75(s, 2H); 6.85(d, 2H); 6.90(d, 1H); 7.20(d, 1H); 7.50(d, 2H); 8.07(s, 1H)
15	451 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 4.05(t, 2H); 4.35(d, 2H); 5.20(s, 2H); 6.85(d, 2H); 6.88(d, 1H); 7.20(d, 1H); 7.41(s, 5H); 7.50(d, 2H); 8.08(s, 1H)
20	Not recorded	1.15-1.45(m, 5H); 1.65-1.95(m, 5H); 2.95(bt, 2H); 3.50(q, 2H); 3.55-3.80(m, 6H); 4.05(t, 2H); 4.3(t, 4H); 4.36(d, 2H); 6.85(d, 2H); 6.90(d, 2H); 7.2(d, 1H); 7.45(d, 2H); 8.05(s, 1H)
21	Not recorded	1.15-1.45(m, 5H); 1.65-1.95(m, 5H); 3.0(bt, 2H); 4.05(t, 2H); 4.22(q, 2H); 4.30(bd, 2H); 4.83(q, 2H); 6.90(d, 2H); 7.04(d, 1H); 7.20(d, 1H); 7.53(d, 2H); 8.05(s, 1H)
22	303 (M+1)	2.2(m, 2H); 2.26(2xs, 9H); 2.99(t, 2H); 3.81(t, 2H); 3.94(s, 3H); 5.87(s, 1H); 7.23(s, 2H); 7.95(s, 1H)
23	329 (M+1)	2.2(m, 2H); 2.26(2xs, 9H); 2.99(t, 2H); 3.81(t, 2H); 4.65(m, 2H); 5.2-5.4(m, 2H); 6.0-6.15(m, 1H); 5.87(s, 1H); 7.23(s, 2H); 8.01(s, 1H)
24	Not recorded	1.3(t, 3H); 2.2(m, 2H); 2.27(2xs, 9H); 2.99(t, 2H); 3.81(t, 2H); 4.18(q, 2H); 5.87(s, 1H); 7.23(s, 2H); 7.96(s, 1H)
25	401 (M+23)	2.2(m, 2H); 2.26(2xs, 9H); 2.99(t, 2H); 3.81(t, 2H); 5.19(s, 2H); 5.87(s, 1H); 7.23(s, 2H); 7.3-7.45(m, 5H); 8.04(s, 1H)

Example 4

The Compound No's 3, 4, 6, 7, 9, 10, 16, 17, 18, 19 from Tables 1 and 2 of the invention were prepared using essentially the same method as described in Example 2 for Compound 2 and were obtained in yields of 61-73%. The compounds were purified by column chromatography on silica gel and characterised by their nuclear magnetic resonance (nmr) spectra and Mass Spectral (MS) data. For convenience the nmr and MS data are recorded in Table 5 below.

Table 5

Compound Number	MS data (ESI)	NMR data: Proton Chemical Shift, δ in ppm ($CDCl_3$)
3	403 (M+1)	1.20-1.40(m, 5H); 1.65-1.95(m, 5H); 2.15(s, 3H); 2.95(t, 2H); 4.05(t, 2H); 4.23(q, 2H); 4.35(d, 2H); 6.85(d, 2H); 6.94(d, 1H); 7.18(d, 1H); 7.60(d, 2H)
4	383 (M+1)	1.20-1.40(m, 5H); 1.65-1.95(m, 5H); 2.15(s, 3H); 2.53(s, 3H); 2.95(t, 2H); 4.05(t, 2H); 4.18(q, 2H); 4.35(d, 2H); 6.89-6.90(2xd, 3H); 7.12(d, 1H); 7.61(d, 2H)
6	463 (M+1)	1.20-1.40(m, 5H); 1.65-1.95(m, 5H); 2.15(s, 3H); 2.95(t, 2H); 3.90(s, 6H); 4.05(t, 2H); 4.22(q, 2H); 4.35(d, 2H); 6.85(s, 2H); 6.90(d, 1H); 7.14(d, 1H)
7	355 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.53(s, 3H); 2.95(t, 2H); 3.93(s, 3H); 4.05(t, 2H); 4.35(d, 2H); 6.88(d, 2H); 6.90(d, 1H); 7.05(d, 1H); 7.51(d, 2H); 8.05(s, 1H)
9	369 (M+1)	1.32(m, 2H); 1.65-1.95(m, 5H); 2.15(s, 3H); 2.49(s, 3H); 2.95(t, 2H); 3.93(s, 3H); 4.06(t, 2H); 4.35(d, 2H); 6.88(d, 2H); 7.15(d, 1H); 7.25(d, 1H); 7.55(d, 2H)
10	403 (M+1)	1.02-1.40(m, 5H); 1.65-1.95(m, 5H); 2.70(q, 2H); 2.95(t, 2H); 3.91(s, 3H); 4.05(t, 2H); 4.35(d, 2H); 6.82(d, 2H); 6.88(d, 1H); 7.15(d, 1H); 7.55(d, 2H)

16	375 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 3.93(s, 3H); 4.03(t, 2H); 4.35(d, 2H); 6.85(d, 2H); 7.03(d, 1H); 7.12(d, 1H); 7.18(d, 1H); 7.28(d, 1H); 8.03(s, 1H)
17	405 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.95(t, 2H); 3.85(s, 3H); 3.93(s, 3H); 4.15(t, 2H); 4.35(d, 2H); 6.85(d, 1H); 6.90(d, 1H); 6.98(dd, 1H); 7.16(d, 1H); 7.23(d, 1H); 8.0(s, 1H)
18	403 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.25(s, 6H); 3.0(t, 2H); 3.85(t, 2H); 3.91(s, 3H); 4.35(d, 2H); 6.92(d, 1H); 7.10(d, 1H); 7.26(s, 2H); 8.0(s, 1H)
19	419 (M+1)	1.30(m, 2H); 1.65-1.95(m, 5H); 2.15(s, 3H); 2.95(t, 2H); 3.90(s, 3H); 3.95(s, 3H); 4.07(t, 2H); 4.35(d, 2H); 6.82(d, 1H); 6.90(d, 1H); 6.99(dd, 1H); 7.14(d, 1H); 7.26(d, 1H)

Example 5

Anti-HRV activity in mammalian cell culture assays

Inhibition of viral cytopathic effect (CPE) and measurement of cytotoxicity

The ability of compounds to suppress virus replication and thereby protect cells from HRV-induced CPE was measured using human embryo lung (MRC-5) and human epidermoid carcinoma of the mouth (KB) cells infected with HRV type 1A and HRV type 2, respectively. Cells grown in 96 well tissue culture plates using conventional mammalian tissue culture medium (such as minimum essential medium) supplemented with fetal calf serum were used in an assay essentially similar to that described by Sidwell and Huffman (Applied Microbiology, 22, 797-801 (1971)). Test compounds were dissolved in 100% anhydrous dimethyl sulfoxide and serially diluted in tissue culture medium. The antiviral potency of the test compounds was assessed by exposing replicate tissue culture wells to a selected dilution series of between 6 and 7 compound concentrations in the presence of sufficient test virus to invoke significant CPE over the course of the assay. Control cells were also exposed to identical

concentrations of compounds in the absence of virus or were infected with virus under the same conditions but in the absence of compounds. Compounds of established anti-HRV efficacy (enviroxime, ribavirin and pirodavir) were assayed by identical procedures in parallel to the test compounds. Tissue culture media were identically supplemented to maintain cell viability and support viral growth while suppressing bacterial growth over the period of the assay (supplements: 2% fetal calf serum, 0.01% sodium bicarbonate, 50 g/ml gentamicin, 5 M magnesium chloride, 10 mM of zinc chloride). The assays were incubated at 37°C in a 5% CO_2 atmosphere until significant CPE was observed by microscopic examination of the untreated, HRV infected control cells (generally between 5 and 8 days). At this time all infected cultures were examined by eye using a light microscope and CPE scored on a scale of 0 (no CPE) to 4 (maximum CPE). Uninfected treated cultures were similarly scored for cytotoxic effects (*eg.* cell enlargement, granularity, rounding, detachment). These scores were used to generate EC_{50} (concentration of compound yielding 50% antiviral efficacy) and CC_{50} (concentration of compound yielding 50% cytotoxicity) values by line regression analysis from plots of compound concentration versus % CPE or % cytotoxicity, respectively. As an alternative to a CC_{50} value, cytoxicity in some cases was expressed as the Minimum Toxic Concentration (MTC). The MTC corresponds to the lowest compound concentration at which cytotoxic effects were observed.

Vital dye staining to measure cell viability was also used to quantify CPE and cytotoxic effects. The vital dye technique was based on either neutral red uptake (Modification of the method of McManus, Appl. Environment. Microbiol., 31, 35-38, 1976) or XXT metabolism. After the assay had been scored by eye with the aid of a microscope, 100 l of neutral red (NR) solution (0.34% NR in phosphate buffered saline (PBS)) was added to each well and mixed gently. The assays were returned to the 37°C incubator for 2 hours to facilitate uptake of the NR by viable cells. The medium/NR mixture was then aspirated from the surface of the cells, which were washed twice with PBS. 0.25 ml of absolute ethanol containing Sorensen's citrate buffer I, was added with gentle mixing and the assays incubated at room temperature in the dark for 30 minutes to

dissolve the NR. NR staining of viable cells was then quantified spectrophotometrically by measuring the colour density of the NR solution using a BioTek EL-309 microplate reader at dual wavelengths of 540 and 405 nm. The differences in the two readings were automatically determined to eliminate background errors. EC_{50} and CC_{50} values were determined by regression analysis matching compound concentration to NR staining. The XTT method involved use of a solution of XTT (1 mg/ml in culture media) which was added to each well and the plates incubated at 37°C for 4 hours. XTT metabolism was measured spectrophotometrically using a similar method to that described above except that the dual wavelengths were 450nm and 650nm. EC_{50} and CC_{50} values were determined by regression analysis using a similar method to that described above.

The results are shown in the table below. Selectivity indices (SI) are the CC_{50} or MTC divided by the EC_{50}.

Table 5

Compound No.	Activity on Rhinovirus Type 2[a]			Activity on Rhinovirus Type 1A		
	EC_{50} (µg/ml)	CC_{50}	SI	EC_{50} (µg/ml)	MTC	SI
1	< 0.001 (< 0.001)	> 1	> 1000	0.0002	> 10.0	>37317
2	0.0002 (0.0003)	10	50,000	<0.000156	>50	>320000
3	0.01 (0.009)	> 1	> 100	0.005	> 10.0	1841
4	0.5 (0.2)	> 1	> 2	0.39	10.00	25.55
6	0.1 (0.1)	> 50	> 500	0.06	> 10.00	> 167.09
20	3	2	1	3.28	> 50.00	> 15.24
22	<0.05	20	>400			

23	<<0.05	17	>340			
24	<0.05	25	>500			
25	0.5	17	34			
Comparative compound:						
Compound No. 110 of US Patent 4,992,433	2 (2)	30	15	12.06	> 50.00	> 4.14
Controls:						
Pirodavir	0.003 (0.004)	> 1	> 300	0.02	> 10.00	555.74
Ribavirin				1.93	98.3	51.03
Enviroxime				0.006	0.49	75.91

[a]The numbers in brackets refer to assessment of the assay using the neutral red dye method.

Example 6

Activity against Enteroviruses in Mammalian cell culture assays

Compounds No 1 and 2 of the invention were tested against other picornaviruses using similar cell based assays to those described in Example 5 above and results are shown in Table 6 below.

Table 6

Compound Number	Activity on Enterovirus 70			Activity on Coxsackie A21		
	EC_{50}(µg/ml)	CC_{50}	SI	EC_{50}(µg/ml)	CC_{50}	SI
1	0.03	>50	>1582	<0.000156	>50	>320000
2	0.00698	4.48	6409	<0.000156	3.43	>21927
Controls:						

Ribavirin	>100	>100	-	>100	>100	-
Enviroxime	0.21	4.11	19.35	0.39	9.55	24.25

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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